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Effect of increased vegetable and fruit consumption on plasma folate and homocysteine concentrations

Rik P. Bogers, Ph.D.[a,*], Pieter C. Dagnelie, Ph.D.[a], Aalt Bast, Ph.D.[b], Marcella van Leeuwen, M.Sc.[a], Jacob D. van Klaveren, M.Sc.[c], and Piet A. van den Brandt, Ph.D.[a]

[a] *Department of Epidemiology, Maastricht University, Maastricht, The Netherlands*
[b] *Department of Pharmacology and Toxicology, Maastricht University, Maastricht, The Netherlands*
[c] *RIKILT Institute of Food Safety, Wageningen, The Netherlands*

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Abstract

Objective: We assessed the effects of an intervention aimed at increasing the consumption of fruits and vegetables on plasma folate and homocysteine concentrations.

Methods: Seventy-one healthy non-smoking women (mean ± SD 41 ± 4 y of age) were randomized to an intervention or a control group. Participants in the intervention group ($n = 36$) received weekly packets containing fruits and vegetables free of charge and were asked to consume a daily amount of ≥200 g of vegetables and two pieces of fruit (the Dutch recommended intake level) over a period of 1 mo. Control subjects did not receive any intervention.

Results: Compared with the control group, reported fruit and vegetable intakes in the intervention group increased by 133 g/d (95% confidence interval [CI] 87–179, $P < 0.001$) for fruits and juice and 64 g/d (95% CI 37–91, $P < 0.001$) for vegetables and estimated folate intake from fruits and vegetables increased by 40 μg/d (95% CI 22–58, $P < 0.001$). However, no effect was observed on plasma folate concentrations (intervention effect 0.3 nmol/L, 95% CI −1.8 to 2.8, $P = 0.77$) or homocysteine concentrations (intervention effect 0.26 μmol/L, 95% CI −0.34 to 0.87, $P = 0.39$).

Conclusion: The results suggest that 4 wk of increased fruit and vegetable consumption to the recommended amounts may be insufficient to change plasma folate and homocysteine concentrations.

Keywords: Randomized controlled intervention study; Micronutrients; Blood; Cardiovascular risk factors; Women

Introduction

Fruits and vegetables contain various micronutrients with potentially favorable effects on human health. Among these micronutrients folate is a vitamin that has received considerable interest because of its effect on public health. A high folate intake by women of child-bearing age is associated with a lower risk of neural tube defects in their newborn children [1]. Moreover, increasing the intake of folate has the potential of lowering high blood concentrations of homocysteine (Hcy), which is associated with a risk of cardiovascular disease [2–4] although evidence for a causal relation is lacking; it has been suggested that Hcy may only be a marker but not a cause of increased vascular disease risk [5,6]. A meta-analysis of 12 randomized controlled trials showed that daily supplementation with folic acid (the form of folate found in vitamin supplements and fortified food) in the range of 0.5–5 mg and with about 0.5 mg of vitamin B12, another determinant of Hcy in blood [7] would be expected to decrease blood Hcy concentrations in Western populations by approximately 25% to 33% [8].

Compared with synthetic folic acid, naturally occurring food folate is less effective in reducing blood Hcy concentrations [9]. Natural sources of folate include fruits and vegetables [10]. In addition, fortification or enrichment of

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* Corresponding author. Tel.: +31-30-274-2150; fax: +31-30-274-4407.

E-mail address: rik.bogers@rivm.nl (R. P. Bogers).

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foods with folate could lead to higher folate intakes. However, this fortification is not allowed in some countries, including the Netherlands. Several intervention studies have been conducted on the potential of fruits and vegetables [9,11–13] or fruits, vegetables, and other folate-rich foods [14–16] to increase folate and decrease Hcy concentrations in blood, with generally favorable results in terms of increasing folate concentrations, but mixed results in terms of lowering Hcy concentrations. These studies compared subjects consuming controlled diets high or low in fruits and vegetables [9,11–13] or provided them with a list of foods rich in folate from which they could choose to increase their folate intake [14–16].

The advice to the public in the Netherlands is to consume two pieces of fruit and 200 g of vegetables per day [17], which is similar to the recommendation to consume at least five servings of fruits and vegetables per day in other Western countries. Little is known about the effects of consuming the recommended amounts of fruits and vegetables irrespective of their folate content, which is closer to the real-life situation and to current dietary recommendations, which do not specifically recommend the consumption of folate-rich fruits and vegetables. Therefore, the present study assessed the effects of an increased consumption of fruits and vegetables to these recommended amounts on plasma folate and Hcy concentrations in a subset of adult women participating in a randomized controlled intervention study on biomarkers of fruit and vegetable consumption and determinants of consumption in adults and children [18–20].

Materials and methods

Study design and study population

Approval for the study was obtained from the medical ethics committee of Maastricht University. Eligible subjects were apparently healthy, non-smoking mothers with primary school-age children who agreed not to use vitamin supplements from 1 mo before the first blood collection until the end of the study period. Participants were recruited by invitation letters distributed to households with children 7–10 y of age by mail and through schools. Recruitment was stopped after >200 women had been enrolled. The response rate to the invitations was 7%.

An initial questionnaire, which was returned by the participants by mail, was sent out in February 2001, including a short (eight-item) food-frequency questionnaire (FFQ) [20]. Participants were randomly allocated to the intervention or control group after prestratification for fruit and vegetable intake (below versus above median) as determined with this FFQ; thus equal numbers of participants with an intake below and above the median consumption were randomized between the two groups. A more extended FFQ, which was used to calculate baseline fruit and vegetable and folate intakes [18], was sent out to intervention and control participants in March 2001. Participants handed in the FFQ at the study center and a fasting venous blood sample was taken. Directly after the 4-wk intervention period, the elaborate FFQ was completed once again and another fasting blood sample taken. The present report is restricted to 71 participants of whom a spare blood sample was available for analysis of folate, Hcy, and vitamin B12. These 71 participants did not differ significantly from the rest of the participants in terms of age, body mass index, and fruit and vegetable consumption at baseline and after intervention (data not shown).

Intervention

Participants in the intervention group were asked to consume a daily amount of ≥200 g of vegetables and ≥ two pieces of fruit (the Dutch recommended intake level) over a period of 1 mo. Packets containing fruits and vegetables sufficient to meet recommended amounts for each family member for 1 wk were delivered weekly to the participants' homes free of charge, together with a newsletter and recipes. In addition, as a means to encourage ample consumption of fruits and vegetables, participants in the intervention group were asked to monitor the number of servings of fruits and vegetables they consumed each day during the intervention period by scoring boxes on a form. Women in the control group received neither fruit and vegetables nor further information on fruit and vegetables.

Dietary assessment

Fruit and vegetable consumption was determined with an FFQ asking detailed information on consumption of specific fruits and vegetables, with a reference period of 1 mo. The FFQ was validated using plasma carotenoids and vitamin C as biomarkers of intake. The FFQ, the validation study, and the procedure used to calculate fruit and vegetable consumption are described elsewhere [18]. Total fruit and vegetable intake was calculated using summary questions on intake of fruits, cooked vegetables and raw vegetables, and fruit and vegetable juice. Potatoes were excluded because these are considered a staple food in the Netherlands. Folate intake from fruits and vegetables was calculated by multiplying the intake of specific fruits, vegetables, and juices (61 items) by their folate content [21]. In the case of combination categories of fruits or vegetables in the FFQ, e.g., cauliflower and broccoli, the corresponding folate content was calculated as a weighed mean folate content of all fruits or vegetables in that category, with the weighing factor calculated from the number of users (women, 22–50 y of age) of a particular fruit or vegetable observed in the third Dutch National Food Consumption Survey [22]. Because the FFQ was not designed to assess the entire diet, total energy intake could not be estimated.

Plasma folate, vitamin B12, and Hcy determination

After an overnight fast, 10 mL of venous blood was collected between 0700 and 1100 h in a plastic tube coated with K_2 ethylene diaminetetra-acetic acid (Becton Dickinson Vacutainer Systems, Plymouth, UK), which was immediately placed on ice in the dark. Subsequently the blood was centrifuged (1000*g*, 10 min, 4°C) within 10 min and plasma was transferred into amber Eppendorf vials and stored at −80°C until analysis. Plasma folate, vitamin B12, and total Hcy concentrations were determined with a competitive immunoassay with direct chemiluminescent technology using an Advia Centaur (Bayer Diagnostics, Tarrytown, NY, USA) at the certified medical laboratory (Dr. Stein & Partners) (Mönchengladbach, Germany). Chemicals were obtained from Bayer Diagnostics. The coefficients of variation for folate were 6.7%, 7.5%, and 14.4% for test samples with concentrations of 28.9, 11.2, and 5.55 nmol/L, respectively. The coefficients of variation for vitamin B12 were 4.5% at 968 pmol/L, 7.1% at 542 pmol/L, and 6.0% at 235 pmol/L. The coefficient of variation for Hcy was 5.5% at 8.7 μmol/L.

Statistical analysis

Statistical analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Standardized regression coefficients were used as measurements of association between plasma concentrations of Hcy and folate, and intake of fruit, vegetables, and folate at baseline. Differences between the control and intervention groups at baseline were tested with the Mann-Whitney U test, and changes from baseline to postintervention within the control and intervention groups were tested with the Wilcoxon signed rank test because most variables were non-normally distributed. Linear regression analysis was used to determine the effect of the intervention on fruit and vegetable intake, folate intake, plasma folate concentrations, and Hcy concentrations after adjusting for baseline values. Because vitamin B12 is a determinant of plasma Hcy concentrations [7], analyses that involved Hcy concentrations were adjusted for plasma vitamin B12 concentrations in cross-sectional analyses and for changes in vitamin B12 concentrations in longitudinal analyses. Regression models were checked for normality of residuals, linearity, and homogeneity of variance. Outliers, defined as observations with an absolute Studentized deleted residual >3.5 were identified as outliers [23] and excluded from the analysis, and models were rerun until no outliers were left. Because of skewed distribution, plasma folate concentrations (but no other blood values) were transformed to their natural logarithm before fitting regression models.

Results

Plasma samples and FFQs were available for a subset of 36 intervention subjects and 35 controls. Participants' ages were 42 ± 4 y (mean ± SD) in the intervention group and 41 ± 3 y in the control group, and mean body mass indexes were 24.5 ± 3.8 kg/m^2 in the intervention group and 23.8 ± 3.0 kg/m^2 in the control group.

At baseline, reported fruit and vegetable consumption was similar in the intervention and control groups. The estimated folate intakes from fruit and vegetables were 113 ± 49 μg/d in the intervention group and 115 ± 48 μg/d in the control group. Plasma folate concentrations were nonsignificantly higher ($P = 0.15$) and plasma Hcy concentrations lower ($P = 0.50$) in the intervention group. Plasma vitamin B12 concentration in the intervention group was 196 ± 82 pmol/L versus 245 ± 98 pmol/L in the control group ($P = 0.016$; Table 1). Participants who consumed more than the median fruit and vegetable intake had statistically non-significantly higher plasma folate concentrations (difference 2.00 nmol/L, $P = 0.33$), higher vitamin B12 concentrations (15 pmol/L; $P = 0.53$), and lower Hcy concentrations (1.19 μmol/L; $P = 0.13$) than did those who consumed less. As listed in Table 2, folate intake calculated from the consumption of fruits and vegetables at baseline was not significantly associated with plasma folate (standardized regression coefficient [RC_s] = 0.21, $P = 0.08$) or Hcy ($RC_s = -0.09$, $P = 0.46$) concentrations. Plasma folate was significantly and positively related to consumption of total vegetables ($RC_s = 0.34$, $P = 0.003$) and cooked vegetables ($RC_s = 0.33$, $P = 0.005$). Plasma Hcy was negatively associated with plasma folate concentrations ($RC_s = -0.30$, $P = 0.013$) and consumption of raw vegetables ($RC_s = -0.24$, $P = 0.045$).

During the intervention period the reported fruit and vegetable consumption in the intervention group increased by 214 ± 155 g/d ($P < 0.000$) and folate intake by 43 ± 46 μg/d ($P < 0.000$) compared with 7 ± 98 g/d ($P = 0.26$) and 2 ± 32 μg/d ($P = 0.86$), respectively, in the control group; the between-group difference (i.e., intervention effect) was statistically significant at $P < 0.001$ for fruit and vegetable consumption and folate intake (Table 1). The observed decreases in plasma folate concentrations of 1.1 ± 8.5 nmol/L ($P = 0.32$) in the intervention group and 1.6 ± 4.9 nmol/L ($P = 0.26$) in the control group were not significantly different between the two groups (intervention effect 0.3 nmol/L, $P = 0.77$). Plasma Hcy concentrations did not change significantly from baseline to postintervention in the intervention group (0.19 ± 1.58 μmol/L, $P = 0.42$) or the control group (−0.30 ± 1.41 μmol/L, $P = 0.28$; intervention effect 0.26 μmol/L, $P = 0.39$). Plasma vitamin B12 concentrations decreased by 14 ± 34 pmol/L in the intervention group ($P = 0.013$) and 17 ± 43 pmol/L in the control group ($P = 0.038$; intervention effect −7 pmol/L, $P = 0.38$).

Discussion

The present study assessed the effects of a 4-wk intervention aimed at increasing the consumption of fruits and

Table 1
Effects of a 4-wk dietary intervention on fruit, vegetable, and folate intake assessed with a food-frequency questionnaire and plasma concentrations of folate and Hcy in 71 healthy women*

	Baseline	Postintervention	Change	Intervention effect (95% CI)†
Fruit and vegetable consumption (g/d)‡				
Intervention group (n = 36)	363 ± 177	577 ± 153	214 ± 155	214 (158–270)
Control group (n = 33)	382 ± 172	375 ± 168	7 ± 98	
Fruit consumption (g/d)§				
Intervention group (n = 36)	218 ± 148	357 ± 109	140 ± 125	133 (87–179)
Control group (n = 34)	239 ± 139	235 ± 132	−4 ± 111	
Vegetable consumption (g/d)				
Intervention group (n = 36)	145 ± 69	220 ± 74	75 ± 69	64 (37–91)
Control group (n = 35)	144 ± 51	154 ± 66	11 ± 48	
Folate intake from fruits and vegetables (μg/d)				
Intervention group (n = 36)	113 ± 49	156 ± 46	43 ± 46	40 (22–58)
Control group (n = 35)	115 ± 48	118 ± 54	2 ± 32	
Plasma folate (nmol/L)				
Intervention group (n = 36)	16.9 ± 8.7	15.7 ± 9.2	−1.1 ± 8.5	0.3 (−1.8 to 2.8)
Control group (n = 35)	14.6 ± 9.3	13.0 ± 5.9	−1.6 ± 4.9	
Plasma Hcy (μmol/L)‖				
Intervention group (n = 35)	9.70 ± 2.56	9.89 ± 2.07	0.19 ± 1.58	0.26 (−0.34 to 0.87)¶
Control group (n = 33)	10.60 ± 4.14	10.30 ± 3.46	−0.30 ± 1.41	
Plasma vitamin B12 (pmol/L)				
Intervention group (n = 36)	196 ± 82	182 ± 80	−14 ± 34	−7 (−24 to 9)
Control group (n = 35)	245 ± 98	228 ± 77	−17 ± 43	

CI, confidence interval; Hcy, homocysteine
* Mean ± SD.
† Intervention effect calculated with linear regression analysis, adjusted for baseline values and relative to control group.
‡ Two outliers excluded.
§ One outlier excluded.
‖ Three outliers excluded.
¶ Also adjusted for changes in plasma vitamin B12 concentrations.

vegetables to at least the recommended intake of 200 g of vegetables and two pieces of fruit per day on plasma folate and Hcy concentrations. The results showed that, although fruit and vegetable consumption in the intervention group during the intervention period increased by 214 g/d compared with the control group, this did not result in increased plasma folate concentrations or in decreased plasma Hcy concentrations.

The present intervention is realistic in the sense that the extra amounts of fruits and vegetables consumed by participants in the intervention group can also be expected to be achieved by the general population. Moreover, the sorts of fruits and vegetables in the packets provided to the participants during the intervention period were selected to represent a variety of fruits and vegetables and were not selected on the basis of their folate content. Although the increase in fruit and vegetable consumption measured by the FFQ was subject to measurement error, a real increase in consumption during the intervention period is likely, because other biomarkers of fruit and vegetable intake (plasma carotenoids and vitamin C) reported in another publication [20] increased from baseline to after intervention; this was the case in the total population and in the subset of participants analyzed in the present study. Further, our data are not biased by supplement use because this was an exclusion criterion. Fortification of foods with folate is not allowed in the Netherlands.

The lack of an intervention effect on plasma folate concentrations contrasts with several previous intervention studies, which showed higher blood folate concentrations in

Table 2
Relations between plasma folate and homocysteine concentrations, and dietary intake of fruits, vegetables, and folate from fruits and vegetables in 71 healthy women at baseline*

	Plasma folate	Plasma Hcy
Fruit and vegetable consumption	0.21 (P = 0.09)	−0.12 (P = 0.31)†
Fruit consumption	0.11 (P = 0.38)	−0.06 (P = 0.61)†
Vegetable consumption	0.34 (P = 0.003)	−0.22 (P = 0.08)†
Cooked vegetables	0.33 (P = 0.005)	−0.09 (P = 0.45)†
Raw vegetables	0.14 (P = 0.25)	−0.24 (P = 0.045)†
Folate intake from fruit and vegetables	0.21 (P = 0.08)	−0.09 (P = 0.46)†
Plasma folate	—	−0.30 (P = 0.013)†
Plasma vitamin B12	0.12 (P = 0.32)	−0.06 (P = 0.59)†‡

Hcy, homocysteine
* Numbers are standardized regression coefficients, which were obtained using univariate models for plasma folate and models adjusted for plasma vitamin B12 concentrations for plasma Hcy.
† One outlier excluded.
‡ Controlled for plasma folate concentrations.

subjects consuming diets high in fruits and vegetables or diets high in folate than in subjects consuming normal diets or diets low in fruits and vegetables or in folate [9,11–16]. Several potential explanations can be given for this contrast.

The first explanation is the duration of the intervention period in the present study. Most comparable to our study is a study by Ashfield-Watt et al. [16], in which an additional folate intake of 50 μg/d for a period of 4 mo resulted in an increase in plasma folate concentrations of 2.76 nmol/L in 41 subjects consuming folate from natural sources compared with a control group. Because in our study an intervention effect on folate intake of 40 μg/d was estimated, the short intervention period of 4 wk is the most likely explanation for a lack of an effect on plasma folate and Hcy concentrations. The findings therefore suggest that in situations in which a rapid increase in plasma folate concentrations is desirable, e.g., during pregnancy, only increasing intake of fruit and vegetables without folic acid supplementation may not be sufficient.

Second, the modest increase in folate intake may be an additional explanation. Previous studies incorporated higher contrasts in fruit and vegetable or folate intake (approximately 100–400 μg/d) between control and intervention groups than did our study [9,13,14], and in some studies the period of enhanced folate intake also was longer than in our study (approximately 400 μg/d higher folate intake in the intervention group for 5–12 wk) [11,12,15]. Additional folate intake in the present study may have been limited by non-compliance or high consumption of fruit and vegetables at baseline. Compliance during the intervention period in our study was not 100%: almost 60% of participants in the intervention group reported having eaten the recommended amount of 200 g/d of vegetables, and almost 80% reported having two pieces of fruit a day during the intervention period. However, excluding non-compliers did not essentially alter our results. Further, baseline fruit and vegetable intake, excluding juice, was high (i.e., 300 g/d in the control group and 304 g/d in the intervention group) compared with the intake of 230 g/d in women 20–49 y of age as measured in the third Dutch National Food Consumption Survey [24]. In addition, plasma folate concentrations in our study population may have been less responsive to an increased folate intake because the mean plasma folate concentration at baseline in the intervention group was relatively high (16.9 ± 8.7 nmol/L) compared with a mean serum folate concentration of 12.2 ± 5.4 nmol/L observed in a subsample of women 20–49 y of age who participated in the second Dutch National Food Consumption Survey [25]. The fruit and vegetable intake at baseline may have been high because the participants were a self-selected group of relatively health-conscious women, as suggested by the relatively low response rate.

Third, plasma folate concentrations unexpectedly decreased (although not statistically significantly) from baseline to after intervention in the intervention and control groups. Factors other than dietary folate intake may have caused this decrease. McKinley et al. [26] reported a seasonal variation in serum folate concentrations independent of dietary folate intake, with highest values observed at the end of winter (February) and lowest values at the end of spring (May). Because the first blood sample in the present study was drawn in spring (March) and the second in April, it is possible that the observed decreasing folate concentrations in the course of the study can be ascribed to seasonal variance. Alternatively, the folate content in fruits and vegetables might have been subject to seasonal variation. However, the nutritional database we used did not provide information on seasonal variation.

Fourth, poor bioavailability of folate may have attenuated the effect of an increased folate intake on plasma folate concentrations. Although it is clear that the bioavailability of naturally occurring folate is incomplete [27] and that preparation [28,29] and cooking methods [30] of vegetables affect bioavailability, we cannot be sure how this affected our findings. Therefore, the bioavailability of folate from different fruits and vegetables and the effect of cooking deserve closer attention.

Fifth, it has to be noted that we only assessed folate intake from fruit and vegetables. Other sources of folate in the Netherlands include potatoes, bread, and dairy products [25]. Although the participants did not report changes in their potato consumption during the intervention period (data not shown), we cannot exclude that the higher intake of fruits and vegetables in the intervention group could have been associated with a decrease in an intake of bread and dairy products to compensate for the extra consumption of fruit and vegetables.

The fact that we did not find an intervention effect on plasma Hcy concentrations is not unexpected given the observation that plasma folate concentrations also remained unaffected. Previous results regarding the effect of a high folate intake on blood Hcy concentrations are mixed. Four studies [9,12–14] showed differences in blood concentrations of Hcy between subjects consuming diets high in fruits and vegetables or dietary (natural occurring) folate and subjects consuming their usual diet or diets low in fruits and vegetables or in folate, whereas three studies [11,15,16] did not. It may be noted that, even if Hcy levels can be lowered by increasing folate intake, its effect on the risk of cardiovascular disease is currently under debate, because in recent trials reductions in plasma Hcy levels had no favorable effects on blood markers of inflammation, endothelial dysfunction, or hypercoagubility [5,6]. This suggests that Hcy is just a marker of vascular risk and not a causal factor or that high Hcy levels may cause cardiovascular disease through mechanisms other than those mentioned.

In accordance with previous studies [11,12,16] we found a negative association between plasma Hcy and folate concentrations at baseline. However, plasma Hcy concentrations were not associated with vitamin B12 concentrations. Although previous studies did show a relation between Hcy and vitamin B12 concentrations, it was generally lower than

that between Hcy and folate concentrations [11,12,16]. The lack of a significant relation between plasma vitamin B12 concentrations and Hcy at baseline in the present study makes it unlikely that the lower baseline B12 levels in the intervention group negatively influenced the effect of the intervention.

Conclusion

Plasma folate or Hcy concentrations in healthy nonsmoking women did not change after fruit and vegetable consumption was increased to the recommended amounts for 1 mo. Potential explanations for the lack of an intervention effect include the relatively short intervention period, a relatively modest increase in dietary folate intake, and a high plasma folate concentration at baseline in the intervention group.

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